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=> e chan lily/au

E1	1	CHAN LILLIAN Y Y/AU
E2	3	CHAN LILLIANE M/AU
E3	25 -->	CHAN LILY/AU
E4	1	CHAN LILY L/AU
E5	2	CHAN LIN W N/AU
E6	37	CHAN LINDA/AU
E7	14	CHAN LINDA S/AU
E8	1	CHAN LINDA Y L/AU
E9	1	CHAN LINDY L/AU
E10	1	CHAN LINDY L T/AU
E11	3	CHAN LING/AU
E12	7	CHAN LING LING/AU

=> s e3-e4

L1 26 ("CHAN LILY"/AU OR "CHAN LILY L"/AU)

=> dup rem l1

PROCESSING COMPLETED FOR L1

L2 17 DUP REM L1 (9 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 17 ANSWERS - CONTINUE? Y/(N):y

L2 ANSWER 1 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1  
AN 2000:364310 BIOSIS  
DN PREV200000364310

TI Two-dimensional electrophoresis map of the human hepatocellular carcinoma cell line, HCC-M, and identification of the separated proteins by mass spectrometry.

AU Seow, Teck Keong; Ong, Shao-En; Liang, Rosa C. M. Y.; Ren, Ee-Chee; Chan, Lily; Ou, Keli; Chung, Maxey C. M. (1)

CS (1) Bioprocessing Technology Center, Clinical Research Center, National University of Singapore, Block MD 11 Level 5, Singapore, 119260 Singapore

SO Electrophoresis, (May, 2000) Vol. 21, No. 9, pp. 1787-1813. print. ISSN: 0173-0835.

DT Article

LA English

SL English

AB Currently, one of the most popular applications of proteomics is in the area of cancer research. In Africa, Southeast Asia, and China, hepatocellular carcinoma is one of the most common cancers, occurring as one of the top five cancers in frequency. This project was initiated with the purpose of separating and identifying the proteins of a human hepatocellular carcinoma cell line, HCC-M. After two-dimensional gel electrophoresis separation, silver staining, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analyses, tryptic peptide masses were searched for matches in the SWISS-PROT and NCBI nonredundant databases. Approximately 400 spots were analyzed using this approach. Among the proteins identified were housekeeping proteins such as alcohol dehydrogenase, alpha-enolase, asparagine synthetase, isocitrate dehydrogenase, and glucose-6-phosphate 1-dehydrogenase. In addition, we also identified proteins with expression patterns that have been postulated to be related to the process of carcinogenesis. These include 14-3-3 protein, annexin, prohibitin, and thioredoxin peroxidase. This study of the HCC-M proteome, coupled with similar proteome analyses of normal liver tissues, tumors, and other hepatocellular carcinoma cell lines, represents the first step towards the establishment of protein databases, which are valuable resources in studies on the differential protein expressions of human hepatocellular carcinoma.

L2 ANSWER 2 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 2

AN 2000:337702 BIOSIS

DN PREV200000337702

TI Cloning and expression of immunoreactive antigens from Mycobacterium tuberculosis.

AU Lim, Renee Lay Hong (1); Tan, Li Kiang; Lau, Wai Fun; Chung, Maxey Ching Ming; Dunn, Roseanne; Too, Heng Phon; Chan, Lily

CS (1) Bioprocessing Technology Centre, National University of Singapore, 10 Kent Ridge Crescent, 5th Floor, MD11, Singapore, 119260 Singapore

SO Clinical and Diagnostic Laboratory Immunology, (July, 2000) Vol. 7, No. 4, pp. 600-606. print. ISSN: 1071-412X.

DT Article

LA English

SL English

AB Four immunoreactive proteins, B.4, B.6, B.10, and B.M, with molecular weights ranging from 16,000 to 58,000, were observed from immunoblots of Mycobacterium tuberculosis total lysates screened with sera from individuals with active tuberculosis. These proteins were identified from microsequence analyses, and genes of proteins with the highest homology were PCR amplified and cloned into the pQE30 vector for expression studies. In addition, a 37.5-kDa protein, designated C17, was identified from a phage expression library of M. tuberculosis genomic DNA. Preliminary immunoblot assays indicated that these five resultant recombinant proteins could detect antibodies in individuals with active pulmonary and extrapulmonary tuberculosis. The overall ranges of sensitivities, specificities, positive predictive values, and negative predictive values for the recombinant antigens were 20 to 58, 88 to 100, 69 to 100, and 56 to 71%, respectively. The B.6 antigen showed preferential reactivity to antibodies in pulmonary compared to nonpulmonary tuberculosis serum specimens. All of these recombinant antigens demonstrated potential for serodiagnosis of tuberculosis.

L2 ANSWER 3 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS

AN 2000:159327 BIOSIS  
DN PREV200000159327  
TI Clinical, pubertal and psychosocial characteristics of adolescents with congenital HIV infection.  
AU Dunn, Ann-Margaret (1); O'Keefe, Kate (1); Chan, Lily (1); Johann-Liang, Rosemary (1)  
CS (1) Program for Children and Adolescents with AIDS, New York-Presbyterian Hospital, New York, NY USA  
SO Pediatric Research., (April, 2000) Vol. 47, No. 4 Part 2, pp. 4A.  
Meeting Info.: Joint Meeting of the Pediatric Academic Societies and the American Academy of Pediatrics. Boston, Massachusetts, USA May 12-16, 2000 American Academy of Pediatrics  
. ISSN: 0031-3998.  
DT Conference  
LA English  
SL English

L2 ANSWER 4 OF 17 CAPLUS COPYRIGHT 2001 ACS

AN 1999:396746 CAPLUS

DN 131:212834

TI Searching for dominant linear antigenic region of hepatitis B surface antigen with human sera against phage-displayed random peptide library

AU Yao, Zhi-Jian; Ong, Lay-Hian; Chan, Lily; Chung, Maxey C. M.

CS Bioprocessing Technology Center, National University of Singapore, Singapore, 119260, Singapore

SO Pept. Proc. Am. Pept. Symp., 15th (1999), Meeting Date 1997, 775-776.

Editor(s): Tam, James P.; Kaumaya, Pravin T. P. Publisher: Kluwer, Dordrecht, Neth.

CODEN: 67UCAR

DT Conference

LA English

AB It is suggested that by using Igs isolated from the patient's body fluids to screen against the repertoire of a random peptide library, the resulting binding peptide sequence(s) that correspond to an epitope or a mimotope of the pathogenic protein would be identified. Here, a recombinant HBsAg soln. was incubated on a polystyrene surface of a Petri dish and the "mono-specific" Ig was purified by elution; 10 .mu.g of recombinant HBsAg was sufficient for prep. the ligate used in 3 rounds of biopanning. Several peptides were synthesized to compare their binding with Ig; to increase sensitivity, all of the peptides were synthesized as their branched form. The results indicate that the cysteine-rich region of the protein was essential for forming the antigenic determinant. In a panel of peptides, in which each amino acid was successfully replaced by alanine, about 85% of the binding affinity was lost when each of the residues 121/124 (cysteine) or 120 (the flanking proline) had been replaced. The mapping results focused directly on the region of residues 110-150, which had been suggested as the major antigenic structure of HBsAg by other approaches in the past 20 yr, thus validating the combinatorial peptide library method which can result in a higher probability of locating the most dominant Ig binding site of a protein.

RE.CNT 5

RE

(1) Rost, B; Methods in Enzymology 1996, V266, P525 CAPLUS

(2) Scott, J; Science 1990, V249, P386 CAPLUS

(3) Tam, J; Proc Natl Acad Sci USA 1988, V85, P5409 CAPLUS

(4) Yao, Z; Int J Peptide Protein Res 1996, V48, P477 CAPLUS

(5) Yao, Z; Protein Chem 1995, V14, P161 CAPLUS

L2 ANSWER 5 OF 17 CAPLUS COPYRIGHT 2001 ACS

AN 1999:474867 CAPLUS

DN 131:291130

TI Photoinduced particulate matter in a parenteral formulation for bisnafide, an experimental antitumor agent

AU Rubino, Joseph T.; Chan, Lily L.; Walker, Joanne T.; Segretario, James; Everlof, J. Gerry; Hussain, Munir A.

CS DuPont Pharmaceuticals Company, Experimental Station, Wilmington, DE, 19880-0400, USA

SO Pharm. Dev. Technol. (1999), 4(3), 439-447

CODEN: PDTEFS; ISSN: 1083-7450

PB Marcel Dekker, Inc.

DT Journal

LA English

AB This paper assesses the cause of particulate formation in vials of the exptl. antitumor agent bisnafide and investigates pharmaceutical techniques to reduce the no. of particulates in the product. Soln. prepn. and particulate isolation were performed under Class 100 laminar air flow. Reversed-phase HPLC and IR microscopy were used to characterize drug and isolated particulate matter, whereas a Hiac particle counter was used to quantify the particulate matter. Particulate matter was obsd. following agitation of the drug solns. and was found to be assocd. with specific lots of drug substance. HPLC of the isolated particulate matter indicated that the particulates consisted largely of bisnafide and impurities that were identified as the products of photodegrdn., confirmed to be the result of the photolytic cleavage of bisnafide to form a poorly sol. aldehyde. The aldehyde may, in turn, interact with bisnafide mols. to form the particulate matter as suggested by the obsd. pH-dependent reversibility of the particulate phenomenon. The particulate matter could be reduced by protecting solns. of bisnafide from light during chem. synthesis and prodn. of the dosage form and, alternatively, by reducing the soln. pH to 3.0 or less, addn. of surfactants below their crit. micelle concn., and removal of impurities by froth flotation of the bisnafide solns.

RE.CNT 9

RE

- (1) Adamson, A; Physical Chemistry of Surfaces 4th ed 1982
- (2) Chatterji, D; J Pharm Sci 1978, V67(4), P526 CAPLUS
- (3) Digenis, G; J Pharm Sci 1994, V83(7), P915 CAPLUS
- (6) Moore, D; J Pharm Pharmacol 1983, V35(8), P489 CAPLUS
- (7) Raghavan, K; Pharm Dev Technol 1996, V1(3), P231 CAPLUS

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L2 ANSWER 6 OF 17 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 3

AN 1998:681973 CAPLUS

DN 129:329703

TI Dengue virus peptides and methods

IN Chan, Lily; Guan, Ming

PA Genelabs Diagnostics Pte. Ltd., Singapore

SO U.S., 21 pp.

CODEN: USXXAM

DT Patent

LA English

FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5824506	A	19981020	US 1994-290268	19940815

AB Peptide antigens derived from the dengue virus type-2 glycoprotein NS1 are provided. The peptide antigens are specifically immunoreactive with sera from individuals infected with the dengue virus. The antigens are useful as diagnostic tools in detg. whether an individual has been or is infected with dengue virus, and for discriminating between infection with dengue virus and infection with related flaviviruses. The antigens are also useful in vaccine comps. for immunizing individuals against infection with the dengue virus.

L2 ANSWER 7 OF 17 USPATFULL

AN 1998:19572 USPATFULL

TI HIV-1/HIV-2 viral detection kit and method

IN Chan, Lily, Singapore, Singapore

Sum, Yoke Wah, Jurong Town, Singapore

Yin, May Fong, Singapore, Singapore

Lim, Lee Fang, Singapore, Singapore

PA Genelabs Diagnostics Pte Ltd., Singapore, Singapore (non-U.S. corporation)

PI US 5721095 19980224

AI US 1995-486837 19950607 (8)

RLI Continuation of Ser. No. US 1994-285880, filed on 4 Aug 1994 which is a continuation of Ser. No. US 1993-68618, filed on 26 May 1993 which is a continuation of Ser. No. US 1992-912220, filed on 10 Jul 1992 which is a

DT Utility  
EXNAM Primary Examiner: Woodward, Michael P.; Assistant Examiner: Brumback, Brenda  
LREP Dehlinger & Associates  
CLMN Number of Claims: 6  
ECL Exemplary Claim: 1  
DRWN 10 Drawing Figure(s); 5 Drawing Page(s)  
LN.CNT 776

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A method for producing an improved solid phase antigenic reagent useful in an immunoassay for detecting antibodies specific for a virus, such as the human immunodeficiency virus, is disclosed which comprising the addition to a natural viral lysate a synthetic or recombinant viral protein or peptide. Also provided is an improved immunoassay utilizing the solid phase antigenic reagent.

L2 ANSWER 8 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 4

AN 1998:405185 BIOSIS

DN PREV199800405185

TI An immunogenic epitope of Chlamydia pneumoniae from a random phage display peptide library is reactive with both monoclonal antibody and patients sera.

AU Naidu, Brindha R.; Ngeow, Yun-Fong; Wang, Lin-Fa; **Chan, Lily**; Yao, Zhi-Jian; Pang, Tikki (1)

CS (1) Inst. Postgrad. Studies Res., Univ. Malaya, 50603 Kuala Lumpur Malaysia

SO Immunology Letters, (June, 1998) Vol. 62, No. 2, pp. 111-115. ISSN: 0165-2478.

DT Article

LA English

AB Random 15-mer peptides displayed on filamentous phages were screened in binding studies using a Chlamydia pneumoniae-specific monoclonal antibody (RR-402) and affinity-purified, polyclonal sera from patients seropositive for C. pneumoniae infections by the microimmunofluorescence (MIF) test. One 15-mer epitope, epitope Cpn15A (LASLCNPKPSDAPVT) was identified in both the monoclonal and polyclonal screenings, and showed higher ELISA reactivity with C. pneumoniae MIF-positive sera compared to patients with other chlamydial infections, non-chlamydial respiratory infections and normal healthy sera (MIF-negative). Interestingly, epitope Cpn15A also showed significant (52%) amino acid sequence homology to the 56 kDa type-specific antigen of Rickettsia tsutsugamushi, a protein implicated in the virulence of this organism.

L2 ANSWER 9 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 5

AN 1998:206006 BIOSIS

DN PREV199800206006

TI Identifying antigenic region of hepatitis B surface antigen by patient's serum with random peptide library.

AU Yao, Zhi-Jian (1); Ong, Lay-Hain; **Chan, Lily**; Chung, Maxey C. M.

CS (1) Bioprocessing Technol. Cent., Natl. Univ. Singapore, Singapore 119260 Singapore

SO Protein and Peptide Letters, (Feb., 1998) Vol. 5, No. 1, pp. 33-40. ISSN: 0929-8665.

DT Article

LA English

AB By screening with random peptide library against human anti-HBsAg antibody, a dominant antibody-binding region was noted. Through peptide synthesis and binding tests, a peptide, corresponding to residues 107-126 and coinciding with a predicted loop region, has been proved to exhibit strong binding capability and the binding could be competitively inhibited by HBsAg. Subsequently, the contributions of each amino acid, sited on this segment were further investigated by alanine scanning.

L2 ANSWER 10 OF 17 CAPLUS COPYRIGHT 2001 ACS

AN 1996:386163 CAPLUS

DN 125:56203

TI Helicobacter pylori diagnostic methods and kits

IN **Chan, Lily**; Moeckli, Randolph; Chin, Daria Foong Yun

PA Genelabs Diagnostics Pte Ltd., Singapore

SO PCT Int. Appl., 20 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9612965	A1	19960502	WO 1995-IB1028	19951019
	W:	AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN			
	RW:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
	AU 9538143	A1	19960515	AU 1995-38143	19951019
PRAI	US 1994-326638		19941020		
	WO 1995-IB1028		19951019		

AB The invention describes an assay for detecting *Helicobacter pylori* infection. The assay is intended for the detection of infection with *Helicobacter pylori* and for the monitoring of the status of infection following treatment. The assay involves an immunoblot for biol. fluid samples and includes a kit in which *Helicobacter pylori* antigen is immobilized on a membrane support. Also provided is a method for diagnosing disease assocd. with *Helicobacter pylori* infection.

L2 ANSWER 11 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 6

AN 1996:23318 BIOSIS

DN PREV199698595453

TI Enhanced specificity of truncated transmembrane protein for serologic confirmation of human T-cell lymphotropic virus type 1 (HTLV-1) and HTLV-2 infections by western blot (immunoblot) assay containing recombinant envelope glycoproteins.

AU Varma, Madhu; Rudolph, Donna L.; Knuchel, Marlyse; Switzer, William M.; Hadlock, Kenneth G.; Velligan, Mark; Chan, Lily; Fong, Steven K. H.; Lal, Renu B. (1)

CS (1) Mailstop G-19, RDB, NCID, CDC, Atlanta, GA 30333 USA

SO Journal of Clinical Microbiology, (1995) Vol. 33, No. 12, pp. 3239-3244. ISSN: 0095-1137.

DT Article

LA English

AB Immunoassays based on the highly immunogenic transmembrane protein of human T-cell lymphotropic virus type 1 (HTLV-1) (protein 21e) are capable of detecting antibodies in all individuals infected with HTLV-1 and HTLV-2. However, because of antigenic mimicry with other cellular and viral proteins, such assays also have a large proportion of false-positive reactions. We have recently identified an immunodominant epitope, designated GD21-I located within amino acids 361 to 404 of the transmembrane protein, that appears to eliminate such false positivity. This recombinant GD21-I protein was used in conjunction with additional recombinant HTLV type-specific proteins and a whole virus lysate to develop a modified Western blot (immunoblot) assay (HTLV WB 2.4). The sensitivity and specificity of this assay were evaluated with 352 specimens whose infection status was determined by PCR assay for the presence or absence of HTLV-1/2 proviral sequences. All HTLV-1-positive (n = 102) and HTLV-2-positive (n = 107) specimens reacted with GD21-I in the HTLV WB 2.4 assay, yielding a test sensitivity of 100%. Furthermore, all specimens derived from individuals infected with different viral subtypes of HTLV-1 (Cosmopolitan, Japanese, and Melanesian) and HTLV-2 (IIa0, a3, a4, IIB1, b4, and b5) reacted with GD21-I in the HTLV WB 2.4 assay. More importantly, HTLV WB 2.4 analysis of 81 PCR-negative specimens, all of which reacted to recombinant protein 21e in the presence or absence of p24 and p19 reactivity in the standard WB assay, showed that only two specimens retained reactivity to GD21-I, yielding an improved test specificity for the transmembrane protein of 97.5%. None of 41 specimens with gag reactivity only or 21 HTLV-negative specimens demonstrated reactivity to GD21-I. In an analysis of additional specimens (n = 169) from different geographic areas for which PCR results were not available, a substantial increase in the specificity of GD21-I detection was demonstrated, with no effect on the sensitivity of GD21-I detection among

specimens from seropositive donors. Thus, the highly sensitive, GD21-I-based HTLV WB 2.4 assay eliminates the majority of false-positive transmembrane results, thereby increasing the specificity for serologic confirmation of HTLV-1 and HTLV-2 infections.

L2 ANSWER 12 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 7  
AN 1995:486711 BIOSIS  
DN PREV199598501011  
TI Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E.  
AU Clayson, Edward T. (1); Myint, Khin Saw Aye; Snitbhan, Rapin; Vaughn, David W.; Innis, Bruce L.; Chan, Lily; Cheung, Peter; Shrestha, Mrigendra P.  
CS (1) USAMC-AFRIMS, 315/6 Rajvithi Rd., Bangkok 10400 Thailand  
SO Journal of Infectious Diseases, (1995) Vol. 172, No. 4, pp. 927-933. ISSN: 0022-1899.  
DT Article  
LA English  
AB Viremia, fecal shedding and antibody responses to hepatitis E virus (HEV) infections are poorly understood. To better characterize HEV infections, these responses were examined in 67 patients with acute markers for hepatitis E who were admitted to the Infectious Disease Hospital in Kathmandu, Nepal in 1993. A single stool and multiple sera from each patient were examined using polymerase chain reaction to detect HEV RNA. Sera were also examined for antibodies to HEV. Viremia, fecal shedding, and IgM and IgG to HEV were detected in 93%, 70%, 79%, and 87% of 67 patients, respectively. Viremia or fecal shedding (or both) were detected in 14 patients from whom IgM and IgG to HEV were not detected. Viremia lasted at least 2 weeks in nearly all subjects and at least 39 days in 1 subject. Our results suggest that viremia is a common occurrence in patients infected with HEV.

L2 ANSWER 13 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 1993:187830 BIOSIS  
DN PREV199395098280  
TI Evaluation of an immunoblot assay for serological confirmation and differentiation of human T-cell lymphotropic virus types I and II.  
AU Roberts, Beverly D. (1); Fount, Steven K. H.; Lipka, James J.; Kaplan, Jonathan E.; Hadlock, Kenneth G.; Reyes, Gregory R.; Chan, Lily; Heneine, Walid; Khabbaz, Rima F.  
CS (1) Retrovirus Diseases Branch, Div. Viral Rickettsial Diseases, Natl. Cent. Infectious Diseases, Cent. Disease Control, Atlanta, GA 30333  
SO Journal of Clinical Microbiology, (1993) Vol. 31, No. 2, pp. 260-264. ISSN: 0095-1137.  
DT Article  
LA English  
AB The confirmation of infection with human T-cell lymphotropic virus type I (HTLV-I) and type II (HTLV-II) currently involves multiple assays. These include Western blot (immunoblot) (WB) and/or radioimmunoprecipitation assay for detection of antibodies to HTLV-specific viral proteins and polymerase chain reaction and/or peptide-based enzyme immunoassays for differentiating between the two viruses. We undertook an evaluation of a modified WB assay that includes native HTLV-I viral proteins from MT-2 cells spiked with an HTLV-I transmembrane glycoprotein (recombinant p21e) and the HTLV-I- and HTLV-II-specific recombinant proteins MTA-1 and K55. The test panel consisted of well-characterized sera from U.S. blood donors, American Indians, intravenous drug users, and patients seen in sexually transmitted disease clinics. Of 158 HTLV-I/II-seropositive serum specimens tested, 156 (98.7%) were confirmed and typed as HTLV-I or HTLV-II. Of 82 HTLV-I/II-seroindeterminate or -seronegative serum specimens, only 1 was classified as HTLV-II positive: the sample had weak gag p19 and strong gag p24 and env p21e reactivity and was radioimmunoprecipitation assay negative for env gp61/68 but polymerase chain reaction positive for HTLV-II. The specificity of the modified WB for confirming and typing serum samples was therefore 100%. We conclude that this WB assay is useful for confirming and typing HTLV infection and can help simplify HTLV-I/II testing algorithms.

L2 ANSWER 14 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 8

AN 1993:274769 BIOSIS  
 DN PREV199396004994  
 TI Isotypic and IgG subclass restriction of the humoral immune responses to human T-lymphotropic virus type-I.  
 AU Lal, Renu B. (1); Buckner, Cindy (1); Khabbaz, Rima F. (1); Kaplan, Jonathan E. (1); Reyes, Gregory; Hadlock, Kenneth; Lipka, Jim; Fount, Steven K. H.; Chan, Lily; Coligan, John E.  
 CS (1) Retrovirus Dis. Branch, Cent. Dis. Control, Atlanta, GA 30333 USA  
 SO Clinical Immunology and Immunopathology, (1993) Vol. 67, No. 1, pp. 40-49. ISSN: 0090-1229.  
 DT Article  
 LA English  
 AB We have investigated the isotypic and IgG subclass profile of the antibody response to HTLV-I structural proteins (gag and env) in patients with HTLV-I-associated myelopathy (HAM; n = 20), adult T-cell leukemia (ATL; n = 15), and HTLV-I-positive asymptomatic carriers (ASY; n = 21). IgG, IgM, and IgA were the predominant antibody responses in all HTLV-I-infected individuals; minimal IgE response was detectable in the HAM and ATL groups. Among the IgG subclasses, IgG-1 was the most predominant antibody detected in responses to HTLV-I antigens, followed by IgG-3 and IgG-2; IgG-4 could not be detected in any patient group. Levels of both IgG-1 and IgG-3 were significantly higher in patients with HAM, when compared to ATL and ASY (P < 0.01 for both comparisons). In addition, Ig isotypes and IgG subclass antibody in patient sera reactive with purified viral proteins and several immunodominant epitopes, represented by synthetic peptides, Gag-1a-102-117, Env-1-191-214, Env-5-242-257, and recombinant proteins, MTA-1-162-209 and r21e-303-440, were examined to delineate specific epitopes responsible for inducing the host immune responses of each isotype and subclass to the structural proteins of HTLV-I. IgG, IgM, and IgA responses were directed against both the gag and env gene products. Among IgG subclasses, the IgG-1 and IgG-3 responses were directed against both the gag (p53, p24, p19, and gag-1a) and env (recombinant MTA-1, r21e, and synthetic Env-1, Env-5) proteins; IgG-2 responses were mainly restricted to gag proteins. The frequency profile of HTLV-I-specific antigen recognition in all four IgG subclasses were similar in all of the clinical groups. These results further define the fine specificity of anti-HTLV-I immune reaction for understanding the mechanisms of pathogenesis in these individuals and suggest that factors other than the humoral immune responses may be associated with the clinical manifestation of the disease.

L2 ANSWER 15 OF 17 CAPLUS COPYRIGHT 2001 ACS  
 AN 1992:406002 CAPLUS  
 DN 117:6002  
 TI An augmented western blot format and immunoassay for detection of viral antibodies  
 IN Chan, Lily; Sum, Yoke Wah; Yin, May Fong; Lim, Lee Fang  
 PA Diagnostic Biotechnology, Inc., USA  
 SO PCT Int. Appl., 33 pp.  
 CODEN: PIXXD2  
 DT Patent  
 LA English  
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9203579	A1	19920305	WO 1991-US5831	19910814
	W: AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MC, MG, MN, MW, NO, PL, RO, SD, SE, SU				
	RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GN, GR, IT, LU, ML, MR, NL, SE, SN, TD, TG				
	AU 9188493	A1	19920317	AU 1991-88493	19910814
	EP 564460	A1	19931013	EP 1991-918547	19910814
	EP 564460	B1	19981021		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
	AT 172542	E	19981115	AT 1991-918547	19910814
	US 5721095	A	19980224	US 1995-486837	19950607
PRAI	US 1990-568144		19900816		
	WO 1991-US5831		19910814		
	US 1992-912220		19920710		



US 1993-68618 19930526

US 1994-285880 1994080

AB A method for producing an improved solid phase antigenic reagent useful in an immunoassay for detecting antibodies to a virus, e.g. human immunodeficiency virus (HIV), is disclosed, which comprises addn. to a natural viral lysate a synthetic or recombinant viral protein or peptide. Peptide sequences for use in detection of antibodies to HIV-1 and -2 by the method of the invention are disclosed. Also provided is an improved immunoassay using the solid-phase antigenic reagent. An immunoblot assay which immediately distinguished HIV-1 seropos. samples from HIV-2 seropos. samples used, in addn. to viral antigen lysate, a genetically engineered HIV-1 envelope protein and a HIV-2-specific synthetic peptide from the envelope sequence.

L2 ANSWER 16 OF 17 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 9

AN 1993:28547 BIOSIS

DN PREV199395016747

TI Immune responsiveness to the immunodominant recombinant envelope epitopes of human T lymphotropic virus types I and II in diverse geographic populations.

AU Buckner, Cindy; Roberts, Chester R.; Fount, Steven K. H.; Lipka, James; Reyes, Gregory R.; Hadlock, Kenneth; Chan, Lily; Gongora-Biachi, Renan A.; Hjelle, Brian; Lal, Renu B. (1)

CS (1) Mail Stop G-19, Centers Disease Control, Atlanta, Ga. 303333

SO Journal of Infectious Diseases, (1992) Vol. 166, No. 5, pp. 1160-1163.

ISSN: 0022-1899.

DT Article

LA English

AB The heterogeneity of immune responsiveness to the immunodominant epitopes of human T lymphotropic virus (HTLV) types I (MTA-1-162-209) and II (K-55-162-205) were determined in natural infections with HTLV-I and -II from diverse geographic areas (n = 285). Of the HTLV-I specimens confirmed by polymerase chain reaction (PCR), all North American (n = 37) and Peruvian (n = 19) specimens reacted with MTA-1. Of HTLV-II specimens confirmed by PCR, 44 (96%) of 46 from North American blood donors, 28 (97%) of 29 from native Americans, and all from intravenous drug users (n = 29) reacted with K-55. Specimens from other geographic areas (Peru, 30; Brazil, 4; Mexico, 10; Italy, 5; Somalia, 13; Ethiopia, 17; Japan, 32; and Jamaica, 15) all reacted either with MTA-1 or K-55. By synthetic peptide-based serologic typing, all of these specimens could be typed as HTLV-I or -II. In addition to the direct implications of these findings for diagnostic purposes, these data provide indirect evidence for the conservation of immunodominant HTLV-env epitopes in diverse geographic populations.

L2 ANSWER 17 OF 17 CAPLUS COPYRIGHT 2001 ACS

AN 1982:50489 CAPLUS

DN 96:50489

TI Modulation of human neutrophil oxidative metabolism

AU Chan, Lily

CS Univ. Illinois, Urbana, IL, USA

SO (1981) 195 pp. Avail.: Univ. Microfilms Int., Order No. 8121683

From: Diss. Abstr. Int. B 1981, 42(5), 1814

DT Dissertation

LA English

AB Unavailable

=> e chung maxey/au

E1	8	CHUNG MAX C M/AU
E2	10	CHUNG MAX CHING MING/AU
E3	0 -->	CHUNG MAXEY/AU
E4	47	CHUNG MAXEY C M/AU
E5	5	CHUNG MAXEY CHING MING/AU
E6	1	CHUNG MAXEY CHUNG MING/AU
E7	2	CHUNG MAY/AU
E8	8	CHUNG MAY A/AU
E9	3	CHUNG MAY YANG/AU

E10 1 CHUNG MEAG AN/AU  
E11 3 CHUNG MEE LING/AU  
E12 1 CHUNG MEEOR LEE/AU

=> s e1-e6

L3 71 ("CHUNG MAX C M"/AU OR "CHUNG MAX CHING MING"/AU OR "CHUNG MAXEY  
"/AU OR "CHUNG MAXEY C M"/AU OR "CHUNG MAXEY CHING MING"/AU OR  
"CHUNG MAXEY CHUNG MING"/AU)

=> dup rem l3

PROCESSING COMPLETED FOR L3

L4 36 DUP REM L3 (35 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 36 ANSWERS - CONTINUE? Y/(N):y

L4 ANSWER 1 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1  
AN 2000:364310 BIOSIS  
DN PREV200000364310  
TI Two-dimensional electrophoresis map of the human hepatocellular carcinoma  
cell line, HCC-M, and identification of the separated proteins by mass  
spectrometry.  
AU Seow, Teck Keong; Ong, Shao-En; Liang, Rosa C. M. Y.; Ren, Ee-Chee; Chan,  
Lily; Ou, Keli; **Chung, Maxey C. M.** (1)  
CS (1) Bioprocessing Technology Center, Clinical Research Center, National  
University of Singapore, Block MD 11 Level 5, Singapore, 119260 Singapore  
SO Electrophoresis, (May, 2000) Vol. 21, No. 9, pp. 1787-1813. print.  
ISSN: 0173-0835.  
DT Article  
LA English  
SL English  
AB Currently, one of the most popular applications of proteomics is in the  
area of cancer research. In Africa, Southeast Asia, and China,  
hepatocellular carcinoma is one of the most common cancers, occurring as  
one of the top five cancers in frequency. This project was initiated with  
the purpose of separating and identifying the proteins of a human  
hepatocellular carcinoma cell line, HCC-M. After two-dimensional gel  
electrophoresis separation, silver staining, matrix-assisted laser  
desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS)  
analyses, tryptic peptide masses were searched for matches in the  
SWISS-PROT and NCBI nonredundant databases. Approximately 400 spots were  
analyzed using this approach. Among the proteins identified were  
housekeeping proteins such as alcohol dehydrogenase, alpha-enolase,  
asparagine synthetase, isocitrate dehydrogenase, and glucose-6-phosphate  
1-dehydrogenase. In addition, we also identified proteins with expression  
patterns that have been postulated to be related to the process of  
carcinogenesis. These include 14-3-3 protein, annexin, prohibitin, and  
thioredoxin peroxidase. This study of the HCC-M proteome, coupled with  
similar proteome analyses of normal liver tissues, tumors, and other  
hepatocellular carcinoma cell lines, represents the first step towards the  
establishment of protein databases, which are valuable resources in  
studies on the differential protein expressions of human hepatocellular  
carcinoma.

L4 ANSWER 2 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 2  
AN 2000:337702 BIOSIS  
DN PREV200000337702  
TI Cloning and expression of immunoreactive antigens from Mycobacterium  
tuberculosis.  
AU Lim, Renee Lay Hong (1); Tan, Li Kiang; Lau, Wai Fun; **Chung, Maxey  
Ching Ming**; Dunn, Roseanne; Too, Heng Phon; Chan, Lily  
CS (1) Bioprocessing Technology Centre, National University of Singapore, 10  
Kent Ridge Crescent, 5th Floor, MD11, Singapore, 119260 Singapore  
SO Clinical and Diagnostic Laboratory Immunology, (July, 2000) Vol. 7, No. 4,  
pp. 600-606. print.

ISSN: 1071-412X.  
DT Article  
LA English  
SL English  
AB Four immunoreactive proteins, B.4, B.6, B.10, and B.M, with molecular weights ranging from 16,000 to 58,000, were observed from immunoblots of Mycobacterium tuberculosis total lysates screened with sera from individuals with active tuberculosis. These proteins were identified from microsequence analyses, and genes of proteins with the highest homology were PCR amplified and cloned into the pQE30 vector for expression studies. In addition, a 37.5-kDa protein, designated C17, was identified from a phage expression library of M. tuberculosis genomic DNA. Preliminary immunoblot assays indicated that these five resultant recombinant proteins could detect antibodies in individuals with active pulmonary and extrapulmonary tuberculosis. The overall ranges of sensitivities, specificities, positive predictive values, and negative predictive values for the recombinant antigens were 20 to 58, 88 to 100, 69 to 100, and 56 to 71%, respectively. The B.6 antigen showed preferential reactivity to antibodies in pulmonary compared to nonpulmonary tuberculosis serum specimens. All of these recombinant antigens demonstrated potential for serodiagnosis of tuberculosis.

L4 ANSWER 3 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 2000:518017 BIOSIS  
DN PREV200000518017

TI An anemic patient with phenotypical beta-thalassemic trait has elevated level of structurally normal beta-globin mRNA in reticulocytes.

AU Lim, Sai-Kiang (1); Ali, Azhar bin; Law, Hai Yang; Ng, Ivy; **Chung, Maxey Chung Ming**; Lee, Szu-Hee

CS (1) National University Medical Institutes, National University of Singapore, Singapore Singapore

SO American Journal of Hematology, (November, 2000) Vol. 65, No. 3, pp. 243-250. print.

ISSN: 0361-8609.

DT Article

LA English

SL English

AB Of the numerous beta-thalassemic mutations linked or unlinked to the beta-globin gene, all invariably cause a decrease in or an absence of structurally normal beta-globin mRNA when assayed. Here we report an anemic patient with an elevated alpha-/beta globin synthesis ratio of 2.0 in his reticulocytes. The patient's blood film showed marked red cell anisopoikilocytosis, microcytosis, and hypochromia, consistent with a typical beta-thalassemic trait phenotype. Acid-eluted erythrocytes contained numerous Heinz bodies. Molecular analysis of the patient's reticulocyte mRNA indicated that, compared to normal controls, there was a 3-fold elevation of beta-globin mRNA when assayed by RT-PCR and a 1.5-fold elevation of beta-globin mRNA when assayed by RNA slot blotting. The level of alpha-globin mRNA was normal when compared to that of normal adult controls. Extensive structural analysis of the beta-globin mRNA and gene by sequencing of RT-PCR and PCR products, respectively, did not detect any mutations. Tryptic mapping of purified beta-globin chains also did not show any abnormal tryptic fragments. These data indicated that a relative insufficiency of structurally normal beta-globin mRNA was not a cause of this beta-thalassemic phenotype. Therefore, the lesion that caused this particular thalassemic phenotype is not linked to the beta-globin allele.

L4 ANSWER 4 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 3  
AN 1999:305412 BIOSIS  
DN PREV199900305412

TI Rhodocetin, a novel platelet aggregation inhibitor from the venom of Calloselasma rhodostoma (Malayan Pit Viper): Synergistic and noncovalent interaction between its subunits.

AU Wang, Runhua; Kini, R. Manjunatha; **Chung, Max C. M.** (1)

CS (1) Department of Biochemistry, National University of Singapore, Singapore Singapore

SO Biochemistry, (June 8, 1999) Vol. 38, No. 23, pp. 7584-7593.

ISSN: 0006-2960.

DT Article

LA English  
SL English  
AB A novel platelet aggregation inhibitor, rhodocetin, was purified from the crude venom of Calloselasma rhodostoma. It inhibited collagen-induced platelet aggregation in a dose-dependent manner, with an IC50 of 41 nM. Rhodocetin has a heterodimeric structure with alpha and beta subunits, which could be separated on a nonreducing denaturing gel or reverse-phase HPLC column. Individually neither subunit inhibited platelet aggregation even at 2.0 muM concentration. Titration and reconstitution experiments showed that, when these subunits are mixed to give a 1:1 complex, most of its biological activity was recovered. The reconstituted complex inhibited platelet aggregation with an IC50 of 112 nM, about 3-fold less effective than the native molecule. Circular dichroism analysis revealed that the reconstituted complex had a spectrum similar to that of the native protein. By using surface plasmon resonance studies, we established that the stoichiometry of binding between the two subunits is 1:1 and the subunits interact with a Kd of 0.14 +/- 0.04 muM. The complete amino acid sequences of the alpha (15956.16 Da, 133 residues) and beta (15185.10 Da, 129 residues) subunits show a high degree of homology with each other (49%) and with the Ca2+-dependent lectin-related proteins (CLPs) (typically 29-48%) isolated from other snake venoms. Unlike the other members of the family in which the subunits are held together by an interchain disulfide bond, rhodocetin subunits are held together only through noncovalent interactions. The cysteinyl residues forming the intersubunit disulfide bridge in all other known CLPs are replaced by Ser-79 and Arg-75 in the alpha and beta subunits of rhodocetin, respectively. These studies support the noncovalent and synergistic interactions between the two subunits of rhodocetin. This is the first reported CLP dimer with such a novel heterodimeric structure.

L4 ANSWER 5 OF 36 CAPLUS COPYRIGHT 2001 ACS

AN 1999:396746 CAPLUS

DN 131:212834

TI Searching for dominant linear antigenic region of hepatitis B surface antigen with human sera against phage-displayed random peptide library

AU Yao, Zhi-Jian; Ong, Lay-Hian; Chan, Lily; Chung, Maxey C. M.

CS Bioprocessing Technology Center, National University of Singapore, Singapore, 119260, Singapore

SO Pept. Proc. Am. Pept. Symp., 15th (1999), Meeting Date 1997, 775-776.

Editor(s): Tam, James P.; Kaumaya, Pravin T. P. Publisher: Kluwer, Dordrecht, Neth.

CODEN: 67UCAR

DT Conference

LA English

AB It is suggested that by using Igs isolated from the patient's body fluids to screen against the repertoire of a random peptide library, the resulting binding peptide sequence(s) that correspond to an epitope or a mimotope of the pathogenic protein would be identified. Here, a recombinant HBsAg soln. was incubated on a polystyrene surface of a Petri dish and the "mono-specific" Ig was purified by elution; 10 .mu.g of recombinant HBsAg was sufficient for prepg. the ligate used in 3 rounds of biopanning. Several peptides were synthesized to compare their binding with Ig; to increase sensitivity, all of the peptides were synthesized as their branched form. The results indicate that the cysteine-rich region of the protein was essential for forming the antigenic determinant. In a panel of peptides, in which each amino acid was successfully replaced by alanine, about 85% of the binding affinity was lost when each of the residues 121/124 (cysteine) or 120 (the flanking proline) had been replaced. The mapping results focused directly on the region of residues 110-150, which had been suggested as the major antigenic structure of HBsAg by other approaches in the past 20 yr, thus validating the combinatorial peptide library method which can result in a higher probability of locating the most dominant Ig binding site of a protein.

RE.CNT 5

RE

(1) Rost, B; Methods in Enzymology 1996, V266, P525 CAPLUS

(2) Scott, J; Science 1990, V249, P386 CAPLUS

(3) Tam, J; Proc Natl Acad Sci USA 1988, V85, P5409 CAPLUS

(4) Yao, Z; Int J Peptide Protein Res 1996, V48, P477 CAPLUS

L4 ANSWER 6 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 4  
AN 1999:365755 BIOSIS  
DN PREV199900365755  
TI Amino acid sequence of trocarin, a prothrombin activator from *Tropidechis carinatus* venom: Its structural similarity to coagulation factor Xa.  
AU Joseph, Jeremiah S.; **Chung, Maxey C. M.**; Jeyaseelan, Kandiah;  
Kini, R. Manjunatha  
CS Bioscience Centre, Faculty of Science, National University of Singapore,  
Singapore, 117600 Singapore  
SO Blood, (July, 1999) Vol. 94, No. 2, pp. 621-631.  
ISSN: 0006-4971.  
DT Article  
LA English  
SL English  
AB Among snake venom procoagulant proteins, group II prothrombin activators are functionally similar to blood coagulation factor Xa. We have purified and partially characterized the enzymatic properties of trocarin, the group II prothrombin activator from the venom of the Australian elapid, *Tropidechis carinatus* (rough-scaled snake). Prothrombin activation by trocarin is enhanced by Ca<sup>2+</sup>, phospholipids, and factor Va, similar to that by factor Xa. However, its amidolytic activity on peptide substrate S-2222 is significantly lower. We have determined the complete amino acid sequence of trocarin. It is a 46,515-Dalton glycoprotein highly homologous to factor Xa and shares the same domain architecture. The light chain possesses an N-terminal Gla domain containing 11 gamma-carboxyglutamic acid residues, followed by two epidermal growth factor (EGF)-like domains; the heavy chain is a serine proteinase. Both chains are likely glycosylated: the light chain at Ser 52 and the heavy chain at Asn 45. Unlike other types of venom procoagulants, trocarin is the first true structural homologue of a coagulation factor. It clots snake plasma and thus may be similar, if not identical, to snake blood coagulation factor Xa. Unlike blood factor Xa, it is expressed in high quantities and in a nonhepatic tissue, making snake venom the richest source of factor Xa-like proteins. It induces cyanosis and death in mice at 1 mg/kg body weight. Thus, trocarin acts as a toxin in venom and a similar, if not identical, protein plays a critical role in hemostasis.

L4 ANSWER 7 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS  
AN 1998:460554 BIOSIS  
DN PREV199800460554  
TI Genomic organization of a K<sup>+</sup>-channel toxin gene from sea anemone: A common path of evolution among ion-channel toxin genes.  
AU Gendeh, Germil S.; **Chung, Max C. M.**; Jeyaseelan, Kandiah  
CS Dep. Biochem., Natl. Univ. Singapore, 10 Kent Ridge Crescent, 119260  
Singapore Singapore  
SO Toxicon, (Sept., 1998) Vol. 36, No. 9, pp. 1294.  
Meeting Info.: 12th World Congress on Animal, Plant and Microbial Toxins  
Cuernavaca, Mexico, USA September 21-26, 1997  
ISSN: 0041-0101.  
DT Conference  
LA English

L4 ANSWER 8 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 5  
AN 1998:206006 BIOSIS  
DN PREV199800206006  
TI Identifying antigenic region of hepatitis B surface antigen by patient's serum with random peptide library.  
AU Yao, Zhi-Jian (1); Ong, Lay-Hain; Chan, Lily; **Chung, Maxey C. M.**  
CS (1) Bioprocessing Technol. Cent., Natl. Univ. Singapore, Singapore 119260  
Singapore  
SO Protein and Peptide Letters, (Feb., 1998) Vol. 5, No. 1, pp. 33-40.  
ISSN: 0929-8665.  
DT Article  
LA English  
AB By screening with random peptide library against human anti-HBsAg antibody, a dominant antibody-binding region was noted. Through peptide synthesis and binding tests, a peptide, corresponding to residues 107-126

and coinciding with a predicted loop region, has been proved to exhibit strong binding capability and the binding could be competitively inhibited by HBsAg. Subsequently, the contributions of each amino acid, sited on this segment were further investigated by alanine scanning.

- L4 ANSWER 9 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 6  
AN 1997:221286 BIOSIS  
DN PREV199799513002  
TI Makatoxin I, a novel toxin isolated from the venom of the scorpion *Buthus martensi* Karsch, exhibits nitrenergic actions.  
AU Gong, Jianping; Kini, R. Manjunatha; Gwee, Matthew C. E.;  
Gopalakrishnakone, P. (1); **Chung, Maxey C. M.**  
CS (1) Dep. Anat., Fac. Med., Natl. Univ. Singapore, Lower Kent Ridge Road, Singapore 119260 Singapore  
SO Journal of Biological Chemistry, (1997) Vol. 272, No. 13, pp. 8320-8324. ISSN: 0021-9258.  
DT Article  
LA English  
AB *Buthus martensi* Karsch venom exhibits nitrenergic action in rat anococcygeus muscle (ACM). We have purified a novel toxin, makatoxin I (MkTx I), which exhibits nitrenergic action, to homogeneity from this venom by a combination of gel-filtration, cation-exchange chromatography, and reverse-phase chromatography. Its purity was assessed by capillary electrophoresis and mass spectrometry. Its molecular weight was found to be 7031.71  $\pm$  2.88 as calculated from electrospray mass spectrographic data. The complete amino acid sequence was elucidated by sequencing of reduced and S-pyridylethylated toxin and a carboxyl-terminal peptide, P5564, generated by the cleavage of toxin with endoproteinase Lys-C. The complete sequence of MkTx I is GRDAYIADSENCTYTICALNPYCNDLCTKNGAKSGYCQWAGRYGNACWCIDLDPKVPIRISG SCR. This toxin is composed of 64 amino acid residues and contains 8 half-cystine residues. Structurally, MkTx I has high similarity to Bot I and Bot II when compared with toxins from other scorpion species. The effects of MkTx I on nitrenergic responses were investigated using the rat isolated ACM mounted in Krebs solution (37 degree C, 5% CO-2 in O-2). MkTx I (2  $\mu$ -g/ml) markedly relaxed the carbachol-precontracted ACM; the relaxation was inhibited by the stereoselective inhibitor of nitric oxide synthase, N-omega-nitro-L-arginine methyl ester (50  $\mu$ -M). Thus, MkTx I is the first  $\alpha$ -toxin that can mediate nitrenergic responses in the rat isolated ACM.
- L4 ANSWER 10 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 7  
AN 1997:360156 BIOSIS  
DN PREV199799666559  
TI Proteolytic specificity of rhodostoxin, the major hemorrhagin of *Calloselasma rhodostoma* (Malayan pit viper) venom.  
AU Tan, Nget-Hong (1); Ponnudurai, Gnanajothy (1); **Chung, Maxey C. M.**  
CS (1) Dep. Biochem., Univ. Malaya, Kuala Lumpur Malaysia  
SO Toxicon, (1997) Vol. 35, No. 6, pp. 979-984. ISSN: 0041-0101.  
DT Article  
LA English  
AB The proteolytic specificity of rhodostoxin, the major hemorrhagin from *Calloselasma rhodostoma* (Malayan pit viper) venom was investigated using oxidized B-chain of bovine insulin as substrate. Six peptide bonds were cleaved: Ser-9-His-10, His<sup>10</sup>-Leu-11, Ala-14-Leu-15, Tyr-16-Leu-17, Gly-20-Glu-21 and Phe-24-Phe-25. Deglycosylated rhodostoxin, however, cleaved primarily at Arg-22-Gly-23.
- L4 ANSWER 11 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 8  
AN 1997:203530 BIOSIS  
DN PREV199799502733  
TI Cloning, characterization and expression of a cDNA clone encoding rabbit ubiquitin-conjugating enzyme, E2-32k.  
AU Sun, Binggang; Jeyaseelan, Kandiah; **Chung, Maxey C. M.**; Tan, Tin-Wee; Chock, P. Boon; Teo, Tian-Seng (1)  
CS (1) Dep. Biochem., Fac. Med., Natl. Univ. Singapore, Singapore 119260 Singapore  
SO Biochimica et Biophysica Acta, (1997) Vol. 1351, No. 1-2, pp. 231-238. ISSN: 0006-3002.

DT Article  
LA English  
AB A cDNA clone encoding rabbit E2-32k was obtained by library screening and PCR. The cDNA contains an open reading frame coding for 238 amino acids which shows an overall identity of 81% to human CDC34, the cell cycle-related ubiquitin-conjugating enzyme. A 50% homology to yeast CDC34 within the conserved core domain was also observed. Northern blot analysis indicated that three transcripts existed in all six rabbit tissues examined but their expression levels varied over a wide range. The putative cDNA coding region was highly expressed in *Escherichia coli* as a his-tagged protein which was purified to homogeneity. The ability of this expressed protein to form a thiolester bond with ubiquitin showed that it was functionally active. The ability of this protein to catalyze the conjugation of ubiquitin to histone H2A and H2B was also examined.

L4 ANSWER 12 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 9  
AN 1998:48638 BIOSIS  
DN PREV199800048638  
TI Genomic structure of a potassium channel toxin from *Heteractis magnifica*.  
AU Genh, Gurmil S.; **Chung, Max C. M.**; Jeyasseelan, Kandiah (1)  
CS (1) Dep. Biochem., National Univ. Singapore, 10 Kent Ridge Crescent, 119260 Singapore Singapore  
SO FEBS Letters, (Nov. 24, 1997) Vol. 418, No. 1-2, pp. 183-188.  
ISSN: 0014-5793.  
DT Article  
LA English  
AB We provide information on the gene encoding the K<sup>+</sup> channel toxin, HmK, of the sea anemone *Heteractis magnifica*. A series of DNA amplifications by PCR, which included the amplification of the 5'-untranslated region of the gene, showed that an intron of 402 nucleotides separated the sequence that encodes the matured toxin from the signal peptide sequence. A second 264 nucleotide intron interrupted the 5'-untranslated region of the previously reported HmK cDNA. Two possible transcription-initiation sites were identified by primer extension analysis. Corresponding TATA-box consensus sequences, characteristic of a promoter region, were also located from PCR products of uncloned libraries of adaptor-ligated genomic DNA fragments. The coding region for matured HmK is intronless. The same is also true for other sea anemone toxins reported thus far. More notably, a similar intron-exon organization is present in other ion channel-blocking toxins from scorpions implying that molecules having similar functions share a similar organization at the genomic level suggesting a common path of evolution.

L4 ANSWER 13 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 10  
AN 1997:150419 BIOSIS  
DN PREV199799449622  
TI Role of polyethyleneimine in the purification of recombinant human tumour necrosis factor beta.  
AU Loh, Kean Chong; Yao, Zhi Jian; Yap, Miranda G. S.; **Chung, Maxey C. M.** (1)  
CS (1) Bioprocessing Technol. Cent., Natl. Univ. Singapore, 10 Kent Ridge, Crescent, Singapore 0511 Singapore  
SO Journal of Chromatography A, (1997) Vol. 760, No. 2, pp. 165-171.  
ISSN: 0021-9673.  
DT Article  
LA English  
AB The chromatographic behaviour of recombinant human tumour necrosis factor beta (rhTNF-beta) (pI approx 9.0) during cation-exchange chromatography at pH 7.5 is investigated. Without prior treatment of the *Escherichia coli* cell extract with polyethyleneimine (PEI), very little rhTNF-beta was bound to the column. However, upon addition of 5% PEI (100 µl ml<sup>-1</sup>) to the cell lysate, rhTNF-beta was shown to bind to cation-exchange columns normally. TNF-beta was readily precipitated from the clarified cell extract by 20% ammonium sulphate, but only ca. 25% of this precipitate could be re-solubilized for further purification. However, when 5% PEI was included in the solubilization buffer, the balance of the rhTNF-beta could be recovered. It is proposed that charge interaction between rhTNF-beta and nucleic acids in the cell extract is responsible for both of these anomalous phenomena, and that PEI (a cationic polyelectrolyte) was able to

disrupt this interaction by displacing rhTNF-beta from the charge complex.

L4 ANSWER 14 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 11  
AN 1997:130642 BIOSIS  
DN PREV199799422455  
TI Complete amino acid sequence of an acidic, cardiotoxic phospholipase A-2 from the venom of *Ophiophagus hannah* (King cobra): A novel cobra venom enzyme with "pancreatic loop."  
AU Huang, Min Zhou; Gopalakrishnakone, P. (1); **Chung, Maxey C. M.**; Kini, R. Manjunatha  
CS (1) Dep. Anat., Fac. Med., Univ. Singapore, Lower Kent Ridge Rd., Singapore 119260 Singapore  
SO Archives of Biochemistry and Biophysics, (1997) Vol. 338, No. 2, pp. 150-156.  
ISSN: 0003-9861.  
DT Article  
LA English  
AB A phospholipase A-2 (OHV A-PLA-2) from the venom of *Ophiophagus hannah* (King cobra) is an acidic protein exhibiting cardiotoxicity, myotoxicity, and antiplatelet activity. The complete amino acid sequence of OHV A-PLA-2 has been determined using a combination of Edman degradation and mass spectrometric techniques. OHV A-PLA-2 is composed of a single chain of 124 amino acid residues with 14 cysteines and a calculated molecular weight of 13719 Da. It contains the loop of residues (62-66) found in pancreatic PLA-2s and hence belongs to class IB enzymes. This pancreatic loop is between two proline residues (Pro 59 and Pro 68) and contains several hydrophilic amino acids (Ser and Asp). This region has high degree of conformational flexibility and is on the surface of the molecule, and hence it may be a potential protein-protein interaction site. A relatively low sequence homology is found between OHV A-PLA-2 and other known cardiotoxic PLA-2s, and hence a contiguous segment could not be identified as a site responsible for the cardiotoxic activity.

L4 ANSWER 15 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 12  
AN 1997:453977 BIOSIS  
DN PREV199799753180  
TI Rabbit ubiquitin-activating enzyme E1: cDNA cloning, sequence and expression.  
AU Sun, Binggang; Jeyaseelan, Kandiah; **Chung, Maxey C. M.**; Teo, Tian-Seng (1)  
CS (1) Dep. Biochem., Fac. Med., Natl. Univ. Singapore, Singapore 119260 Singapore  
SO Gene (Amsterdam), (1997) Vol. 196, No. 1-2, pp. 19-23.  
ISSN: 0378-1119.  
DT Article  
LA English  
AB A cDNA clone encoding ubiquitin-activating enzyme E1 has been isolated from a rabbit heart cDNA library and sequenced. The 3.485 kb cDNA contains an open reading frame of 1058 amino acid residues which predicts a protein of approx. 118 kDa. The deduced protein sequence exhibits a very high homology to other ubiquitin-activating enzymes identified in a variety of organisms. Northern blot analysis reveals a single transcript of approx. 3.5 kb in all the rabbit tissues examined. The entire coding region of the rabbit E1 cDNA has been expressed as a his-tagged protein. The recombinant protein has been verified by its ability to cross-react with anti-human E1 antibodies. Ubiquitin thiolester assay shows that the recombinant rabbit E1 protein is functional.

L4 ANSWER 16 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 13  
AN 1996:532145 BIOSIS  
DN PREV199699254501  
TI Stonustoxin is a novel lethal factor from stonefish (*Synanceja horrida*) venom: cDNA cloning and characterization.  
AU Ghadessy, Farid John; Chen, Desong; Kini, R. Manjunatha; **Chung, Maxey C. M.**; Jeyaseelan, Kandiah; Khoo, Hoon Eng (1); Yuen, Raymond  
CS (1) Dep. Biochem., Fac. Medicine, National University Singapore, 10 Kent Ridge Crescent, Singapore 119260 Singapore  
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 41, pp. 25575-25581.  
ISSN: 0021-9258.



DT Article  
LA English  
AB Stonustoxin (SNTX) is a multifunctional lethal protein isolated from venom elaborated by the stonefish, *Synanceja horrida*. It comprises two subunits, termed  $\alpha$  and  $\beta$ , which have respective molecular masses of 71 and 79 kDa. SNTX elicits an array of biological responses both in vitro and in vivo, particularly a potent hypotension that appears to be mediated by the nitric oxide pathway. As a prelude to structure-function studies, we have isolated and sequenced cDNA clones encoding the  $\alpha$ - and  $\beta$ -subunits of SNTX from a venom gland cDNA library. The deduced amino acid sequence of neither subunit shows significant homology with any known protein. Protein sequence alignment does, however, show the subunits to be 50% homologous to each other and implies that they may have arisen from a common ancestor. The subunits of this novel toxin lack typical N-terminal signal sequences commonly found in proteins that are secreted via the endoplasmic reticulum-Golgi apparatus pathway, indicating the possibility of its being secreted by a non-classical pathway, which is not clearly understood. The SNTX subunits have been expressed in *Escherichia coli* as cleavable fusion proteins that cross-react with antibodies raised against the native toxin. To the best of our knowledge, this is the first complete sequence of a fish-derived protein toxin to be reported.

L4 ANSWER 17 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 14  
AN 1996:380626 BIOSIS  
DN PREV199699102982

TI Cloning of the aldehyde reductase gene from a red yeast, *Sporobolomyces salmonicolor*, and characterization of the gene and its product.  
AU Kita, Keiko (1); Matsuzaki, Koji; Hashimoto, Tetsu; Yanase, Hideshi; Kato, Nobuo; **Chung, Max Ching-Ming**; Kataoka, Michihiko; Shimizu, Sakayu  
CS (1) Dep. Biotechnol., Tottori Univ., 4-101 Koyama, Tottori 680 Japan  
SO Applied and Environmental Microbiology, (1996) Vol. 62, No. 7, pp. 2303-2310.  
ISSN: 0099-2240.

DT Article  
LA English  
AB An NADPH-dependent aldehyde reductase (ALR) isolated from a red yeast, *Sporobolomyces salmonicolor*, catalyzes the reduction of a variety of carbonyl compounds. To investigate its primary structure, we cloned and sequenced the cDNA coding for ALR. The aldehyde reductase gene (ALR) comprises 969 bp and encodes a polypeptide of 35,232 Da. The deduced amino acid sequence showed a high degree of similarity to other members of the aldo-keto reductase superfamily. Analysis of the genomic DNA sequence indicated that the ALR gene was interrupted by six introns (two in the 5' noncoding region and four in the coding region). Southern hybridization analysis of the genomic DNA from *S. salmonicolor* indicated that there was one copy of the gene. The ALR gene was expressed in *Escherichia coli* under the control of the *tac* promoter. The enzyme expressed in *E. coli* was purified to homogeneity and showed the same catalytic properties as did the enzyme from *S. salmonicolor*.

L4 ANSWER 18 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 15  
AN 1997:13226 BIOSIS  
DN PREV199799312429

TI Linear epitopes of sperm whale myoglobin identified by polyclonal antibody screening of random peptide library.  
AU Yao, Zhi-Jian (1); Chan, Moey-Chu; Kao, Mandy C. C.; **Chung, Maxey C. M.**  
CS (1) Bioprocessing Technol. Centre, National Univ. Singapore, 10 Kent Ridge Crescent, Singapore 119260 Singapore  
SO International Journal of Peptide & Protein Research, (1996) Vol. 48, No. 5, pp. 477-485.  
ISSN: 0367-8377.

DT Article  
LA English  
AB Distinct enhancement of antibody-specific clones was apparent during the screening against random peptide libraries with antigen-specific polyclonal antibodies. Several sequence motifs obtained from these screenings were homologous with the primary sequence of myoglobin. Two of

these motifs have been confirmed as antigenic determinants by competitive inhibition tests using eight-branched synthetic peptides. One of the peptides has a sequence that corresponds to amino acid residues 42-50, KFDRFKHLK, of the myoglobin sequence. This is a new epitope of myoglobin that is reported for the first time. The epitope is located precisely in the 'turn' or 'loop' region between helices C and D of the crystal structure of myoglobin. The second antibody binding site has a sequence of DIAAKYKELGYQG, and this is located between residues 141-153, which is the C-terminus of myoglobin. This epitope encompassed two linear epitopes of myoglobin, amino acid residues 145-151 and 147-153, that have been reported earlier based on immunochemical characterization of cleavage fragments of the protein. These results clearly indicate that epitope mapping using polyclonal antibodies against random peptide libraries can identify new epitopes precisely, as well as confirm epitopes of myoglobin obtained earlier using established methodologies.

L4 ANSWER 19 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 16  
 AN 1996:519477 BIOSIS  
 DN PREV199699241833  
 TI Improvements on the purification of mannan-binding lectin and demonstration of its Ca-2+-independent association with a C1s-like serine protease.  
 AU Tan, Suet Mien; **Chung, Maxey C. M.**; Kon, Oi Lian; Thiel, Steffen; Lee, Szu Hee; Lu, Jinhua (1)  
 CS (1) Dep. Biochemistry, Natl. Univ. Singapore, 10 Kent Ridge Crescent, Singapore 110260 Singapore  
 SO Biochemical Journal, (1996) Vol. 319, No. 2, pp. 329-332. ISSN: 0264-6021.  
 DT Article  
 LA English  
 AB Mannan-binding lectin (MBL), previously called 'mannan-binding protein' or MBP, is a plasma C-type lectin which, upon binding to carbohydrate structures on micro-organisms, activates the classical pathway of complement. Purification of MBL relies on its Ca-2+-dependent affinity for carbohydrate, but existing methods are susceptible to contamination by anti-carbohydrate antibodies. In the present study a sequential-sugar-elution method has been developed which can achieve a preparation of virtually pure MBL and its associated serine protease (MBL-associated serine protease, MASP) by two steps of affinity chromatography. In further separation of MASP from MBL, it was found that activated MASP was associated with MBL independent of Ca-2+. Since MBL was found to bind to underivatized Sepharose 4B, the MBL-MASP complex was purified using Sepharose 4B and protease inhibitors were included to purify the complex with MASP in its proenzyme form. Analysis of thus purified MBL-MASP complex by gel filtration on a Sephacryl S-300 column at pH 7.8 showed that the proenzyme MASP was also associated with MBL independently of Ca-2+, but that the complex could be disrupted at a low pH (5.0). Therefore the mechanism of MBL-MASP-mediated complement activation appears to be significantly different from the C1-mediated classical pathway.

L4 ANSWER 20 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 17  
 AN 1996:116545 BIOSIS  
 DN PREV199698688680  
 TI Structural studies of a major hemorrhagin (rhodostoxin) from the venom of Calloselasma rhodostoma (Malayan pit viper).  
 AU **Chung, Maxey C. M.** (1); Ponnudurai, Gnanajothy; Kataoka, Michihiko; Shimizu, Sakayu; Tan, Nget-Hong  
 CS (1) Dep. Biochem., Fac. Med., Natl. Univ. Singapore, Singapore 0511 Singapore  
 SO Archives of Biochemistry and Biophysics, (1996) Vol. 325, No. 2, pp. 199-208. ISSN: 0003-9861.  
 DT Article  
 LA English  
 AB The complete amino acid sequence, disulfide linkages, glycosylation sites, and carbohydrate structure of rhodostoxin, the major hemorrhagin from Calloselasma rhodostoma (Malayan pit viper), have been determined. This sequence confirmed the deduced amino acid sequence of the putative hemorrhagic protein encoded by the prorrhodostomin cDNA of C. rhodostoma.

Rhodostoxin contained four disulfide bonds that link Cys19-Cys60, Cys117-Cys198, Cys157-Cys162, and Cys159-Cys165. It is the first four-disulfide proteinase reported among all known venom metalloproteinases, which are either of the two-disulfide or three-disulfide type. Peptide-mapping and dot-blotting experiments showed the presence of two glycopeptides. Subsequent sequencing of these peptides established that the N-glycosylation sites are located at residues 91 and 181 of the amino acid sequence of the matured protein. Mass spectrometric analyses of these glycopeptides showed that they contain an oligosaccharide structure consisting of 4 units of N-acetylglucosamine, 5 units of hexose, 1 unit of fucose, and 2 units of neuraminic acids. The complete carbohydrate structure was then established by 2-D mapping analysis of the pyridylamino-oligosaccharides after hydrazinolysis and pyridyl-lamination of the glycan chains.

- L4 ANSWER 21 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 18  
 AN 1996:332073 BIOSIS  
 DN PREV199699054429  
 TI The complete sequence of a Singapore isolate of odontoglossum ringspot virus and comparison with other tobamoviruses.  
 AU Chng, Chee-Giok; Wong, Sek-Man (1); Mahtani, Parvesh Hariram; Loh, Chiang-Shiong; Goh, Chong-Jin; Kao, Mandy Chai-Chen; **Chung, Maxey Ching-Ming**; Watanabe, Yuichiro  
 CS (1) Dep. Botany, Natl. Univ. Singapore, Singapore 119260 Singapore  
 SO Gene (Amsterdam), (1996) Vol. 171, No. 2, pp. 155-161.  
 ISSN: 0378-1119.  
 DT Article  
 LA English  
 AB The complete sequence of a Singapore isolate of odontoglossum ringspot virus (ORSV-S1) comprises 6609 nucleotides (nt) and four open reading frames (ORFs 1 to 4). The 126/183-kDa RNA-dependent RNA polymerase (RdRp), 33-kDa movement protein (MP) and 18-kDa coat protein (CP) cistrons are located at nt 63-3401/4901, 4807-5718, and 5721-6197 on the genome, respectively. The 5' UTR contains three copies of an 8-base direct repeat and (CAA)-n motifs. Characteristic tRNA-like structure and three consecutive homologous regions were present in the 3' UTR. The genomic RNA and MP of ORSV-S1 are one of the longest among all members of the TOV group. Phylogenetic analysis of all four genes indicates evolutionary divergence, but within each gene there are some degrees of evolutionary convergence. The conserved amino acid sequences in the MP can be used for the classification of tobamoviruses.
- L4 ANSWER 22 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 19  
 AN 1995:390830 BIOSIS  
 DN PREV199598405130  
 TI Analysis of recombinant human tumor necrosis factor beta by capillary electrophoresis.  
 AU Yao, Yi Ju; Loh, Kean Chong; **Chung, Maxey C. M.**; Li, Sam F. Y.  
 (1)  
 CS (1) Dep. Chem., Natl. Univ. Singapore, Kent Ridge Crescent, Singapore 0511 Singapore  
 SO Electrophoresis, (1995) Vol. 16, No. 4, pp. 647-653.  
 ISSN: 0173-0835.  
 DT Article  
 LA English
- L4 ANSWER 23 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 20  
 AN 1995:358136 BIOSIS  
 DN PREV199598372436  
 TI Purification and characterization of two forms of cytochrome b-5 from an arachidonic acid-producing fungus, *Mortierella hygrophila*.  
 AU Kouzaki, Norihiko; Kawashima, Hiroshi; **Chung, Max Ching-Ming**; Shimizu, Sakayu (1)  
 CS (1) Dep. Agricultural Chemistry, Kyoto Univ., Kitashirakawa-Oiwakecho, Sakyo-ku, Kyoto 606 Japan  
 SO Biochimica et Biophysica Acta, (1995) Vol. 1256, No. 3, pp. 319-326.  
 ISSN: 0006-3002.  
 DT Article  
 LA English

AB Two forms of cytochrome b-5 have been purified from the microsomes of an arachidonic acid-producing fungus, *Mortierella hydrophila* FO 5941, after detergent solubilization. They have monomeric molecular masses of about 16 kDa and 19 kDa. Their absorption spectra are similar to those of mammalian cytochrome b-5s. Their amino acid compositions show some similarity to those of mammalian cytochrome b-5s, but the contents of some amino acids (glycine, alanine, aspartic acid + asparagine, glutamic acid + glutamine, arginine, proline, histidine, leucine and lysine) are unique to the cytochrome b-5s of *M. hydrophila*. Some of their internal peptide sequences also show close homology with those of some mammals (approx. 65 to 67%), while some others show no or little homology. The addition of various acyl-CoAs to NADH-reduced microsomes caused an abrupt shutdown of the steady state reduction level of cytochrome b-5s. This indicates the increased utilization of electrons for the desaturation process and may suggest that the cytochrome b-5s of this fungus actually take part in its microsomal desaturation system for polyunsaturated fatty acid biosynthesis as electron carriers.

L4 ANSWER 24 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 21

AN 1995:345753 BIOSIS

DN PREV199598360053

TI Epitope identification by polyclonal antibody from phage-displayed random peptide library.

AU Yao, Zhi-Jian (1); Kao, Mandy C. C.; **Chung, Maxey C. M.**

CS (1) Bioprocessing Technol. Centre, Natl. Univ. Singapore 0511 Singapore

SO Journal of Protein Chemistry, (1995) Vol. 14, No. 3, pp. 161-166.

ISSN: 0277-8033.

DT Article

LA English

AB Screening of bioactive peptides from random peptide libraries using monoclonal antibodies as ligates is an effective method to define epitopes of protein antigens. However, it is thought that polyclonal antibodies might also serve as promising ligates for screening. We illustrate this approach by using recombinant human lymphotoxin (rhLT) polyclonal antibody as a model. The procedure consists in (a) affinity purification of polyclonal antibody to obtain the "monospecific" antibody, (b) screening against a phage-displayed random peptide library using the affinity-purified antibody, (c) plating the enriched phage on agar plates, randomly picking clones, and selecting the positive ones by dot blotting, (d) DNA sequencing of the positive clones and conducting a homology search against the protein sequence databank, and (e) confirming the epitopes by chemical peptide synthesis. By employing this procedure, we identified a dominant epitope RQHPKM, located at residues 15-20 of the human lymphotoxin amino acid sequence. The usefulness of this general procedure is discussed.

L4 ANSWER 25 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 22

AN 1995:206659 BIOSIS

DN PREV199598220959

TI A serotype-specific epitope of dengue virus 1 identified by phage displayed random peptide library.

AU Yao, Zhi-Jian (1); Kao, Mandy C. C.; Loh, Kean-Chong; **Chung, Maxey C. M.**

CS (1) Bioprocessing Technol. Centre, Natl. Univ. Singapore, Singapore

SO FEMS Microbiology Letters, (1995) Vol. 127, No. 1-2, pp. 93-98.

ISSN: 0378-1097.

DT Article

LA English

AB From a panel of monoclonal antibodies of dengue viruses, a serotype-specific epitope of dengue virus 1 was screened from a random peptide library displayed on phage. The epitope was the determinant reactive with monoclonal antibody 15F3-1 that was specific to dengue 1. The screening was monitored by a dot blotting procedure, and after three rounds of screening a consensus motif, HRYSWK, was found. This sequence matches the sequence HKYSWK, corresponding to the amino acid residues 885-890 of polyprotein or residues 111-116 of the non-structural protein 1 of dengue virus serotype 1. The linear epitope was confirmed by testing the antigenicity of chemically synthesized 8-branched peptide.

L4 ANSWER 26 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 23

AN 1995:28749 BIOSIS

DN PREV199598043049

TI The amino acid sequences of two postsynaptic neurotoxins isolated from Malayan cobra (*Naja naja sputatrix*) venom.

AU **Chung, Maxey C. M.** (1); Tan, Nget-Hong; Armugam, Arunmozhiarasi

CS (1) Dep. Biochem., Bioprocessing Technol. Unit, Natl. Univ. Singapore, Kent Ridge Singapore

SO Toxicon, (1994) Vol. 32, No. 11, pp. 1471-1474.

ISSN: 0041-0101.

DT Article

LA English

AB The complete amino acid sequences of two postsynaptic neurotoxins (toxin-3 and toxin-5) isolated from Malayan cobra (*Naja naja sputatrix*) venom were determined by direct automated Edman degradation of peptides obtained from digests with various proteases. Toxin-3 and toxin-5 are both short-chain neurotoxins and their amino acid sequences are highly homologous to *Naja naja atra* and *Naja naja philippinensis* neurotoxin, respectively. Toxin-3 is unique in possessing aspartic acid (D) as the fifth residue, while all other homologous short-chain neurotoxins have asparagine (N) at the corresponding position.

L4 ANSWER 27 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 24

AN 1994:298637 BIOSIS

DN PREV199497311637

TI Continuous epitopes of human lymphotoxin and their topographies.

AU Yao, Zhi-Jian (1); Kao, Mandy C. C.; Loh, Kean-Chong; **Chung, Maxey C. M.**

CS (1) Bioprocessing Technology Unit, Natl. Univ. Singapore, 10 Ken Ridge Crescent, Singapore 0511 Singapore

SO Biochemistry and Molecular Biology International, (1994) Vol. 32, No. 5, pp. 951-959.

DT Article

LA English

AB Human lymphotoxin (hLT or TNF-beta) is a lymphokine that is structurally and functionally related to tumor necrosis factor alpha (TNF-alpha). The continuous epitopes of hLT were located by examining the cross-reaction between rabbit anti-hLT antibody and peptides derived from proteolytic digestion and chemical synthesis. Three antigenic sites, corresponding to residues 40-48, 83-94 and 139-147, of the protein sequence, were located by this approach. Since residues 49-57 also exhibited trace antigenicity, but residues 45-52 displayed no reaction, the whole peptide fragment consisting of residues 40-57 might be necessary for antigenicity. A comparison of the antigenic determinants with the loop structures obtained from X-ray crystallographic studies of hLT showed that all of the epitopes are found on or adjacent to functionally important domains.

L4 ANSWER 28 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 25

AN 1994:457916 BIOSIS

DN PREV199497470916

TI Purification and properties of the L-amino acid oxidase from Malayan pit viper (*Calloselasma rhodostoma*) venom.

AU Ponnudurai, Gnanajothy; **Chung, Maxey C. M.**; Tan, Nget-Hong (1)

CS (1) Dep. Biochem., Univ. Malaya, Kuala Lumpur Malaysia

SO Archives of Biochemistry and Biophysics, (1994) Vol. 313, No. 2, pp. 373-378.

ISSN: 0003-9861.

DT Article

LA English

AB The L-amino acid oxidase of Malayan pit viper (*Calloselasma rhodostoma*) venom was purified to electrophoretic homogeneity. The molecular weight of the enzyme was 132,000 as determined by Sephadex G-200 gel filtration chromatography and 66,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. It is a glycoprotein, has an isoelectric point of 4.4, and contains 2 mol of flavin mononucleotide per mole of enzyme. The N-terminal amino acid sequence of the enzyme was A-D-D-R-N-P-L-A-E-E-F-Q-E-N-N-Y-E-E-F-L. Kinetic studies suggest the presence of a alkyl side-chain binding site in the enzyme and that the

binding site comprises at least four hydrophobic subsites. The characteristics of the binding site differ slightly from those of cobra venom L-amino acid oxidase.

L4 ANSWER 29 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 26  
AN 1994:125413 BIOSIS  
DN PREV199497138413  
TI Rapid purification of recombinant human tumor necrosis factor beta.  
AU Loh, Kean Chong; Yao, Zhi Jian; Yap, Miranda G. S.; **Chung, Maxey C. M. (1)**  
CS (1) Dep. Biochem., Natl. Univ. Singapore, 10 Kent Ridge, Crescent, Singapore 0511 Singapore  
SO Protein Expression and Purification, (1994) Vol. 5, No. 1, pp. 70-75. ISSN: 1046-5928.  
DT Article  
LA English  
AB A rapid and improved method for the purification of recombinant human tumor necrosis factor beta (rhTNF-beta) from Escherichia coli HB 101 cells has been developed. The method utilized sequential steps of polyethylenimine (PEI) and ammonium sulfate precipitation to remove most of the extraneous proteins and nucleic acids from the cell extracts. The final step of purification consisted of DEAE-Sepharose chromatography at pH 7.5 in which rhTNF-beta was eluted with starting buffer. This procedure, when compared to the earlier methods of purification, is highly efficient since we could increase the overall yield of rhTNF-beta and reduce the purification time considerably. The final yield that we obtained from 1 liter of fermentation broth (containing approximately 80 g of wet cells) was 40-50 mg.

L4 ANSWER 30 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 27  
AN 1995:33559 BIOSIS  
DN PREV199598047859  
TI Thermostable N-carbamoyl-D-amino acid amidohydrolase: Screening, purification and characterization.  
AU Ogawa, Jun; **Chung, Max Ching-Ming**; Hida, Shinobu; Yamada, Hideaki; Shimizu, Sakayu (1)  
CS (1) Dep. Agric. Chem., Kyoto Univ., Kitashirakawa, Oiwake-cho, Sakyo-ku, Kyoto 606 Japan  
SO Journal of Biotechnology, (1994) Vol. 38, No. 1, pp. 11-19. ISSN: 0168-1656.  
DT Article  
LA English  
AB A thermostable N-carbamoyl-D-amino acid amidohydrolase was found in the cells of newly isolated bacterium, Blastobacter sp. A17p-4. The bacterium also showed D-specific hydantoinase activity. The N-carbamoyl-D-amino acid amidohydrolase activity of the cells exhibited a temperature optimum at 50-55 degree C, and was stable up to 50 degree C. The N-carbamoyl-D-amino acid amidohydrolase of Blastobacter sp. A17p-4 was purified to homogeneity and characterized. It has a relative molecular weight of about 120,000 and consists of three identical subunits with a relative molecular weight of about 40,000. N-Carbamoyl-D-amino acids having hydrophobic groups served as good substrates for the enzyme. It has been suggested that D-amino acid production from DL-5-substituted hydantoin involves the action of a series of enzymes involved in pyrimidine degradation, namely amide-ring opening enzyme, dihydropyrimidinase, and N-carbamoylamide hydrolyzing enzyme, beta-ureidopropionase. However, the purified enzyme did not hydrolyze beta-ureidopropionate; suggesting that the N-carbamoyl-D-amino acid amidohydrolase coexisting with D-specific hydantoinase, probably dihydropyrimidinase, in Blastobacter sp. A17p-4 is different from beta-ureidopropionase.

L4 ANSWER 31 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 28  
AN 1994:110943 BIOSIS  
DN PREV199497123943  
TI Purification and partial characterization of two cytolysins from a tropical sea anemone Heteractis magnifica.  
AU Khoo, Kong Soo (1); Kam, Wai Kuen (1); Khoo, Hoon Eng (1); Gopalakrishnakone, P.; **Chung, Maxey C. M. (1)**  
CS (1) Dep. Biochem., Natl. Univ. Singapore, 10 Kent Ridge Crescent 0511,

Singapore Singapore  
SO Toxicon, (1993) Vol. 31, 12, pp. 1567-1579.  
ISSN: 0041-0101.  
DT Article  
LA English  
AB Two cytolytins, designed as magnificalytins I and II, were purified from a tropical sea anemone, *Heteractis magnifica* (formerly *Radianthus ritteri*). The purification steps involved Sephadex G-50 and CM-Sepharose chromatography followed by Mono S and Phenyl-Superose Fast Protein Liquid Chromatography. The relative mol. wt of magnificalytins I and II, determined by SDS-PAGE, was approximately 19,000, while their isoelectric points, determined by isoelectric focusing in immobilized pH gradients, were 9.4 and 10.0, respectively. Those toxins were found to have haemolytic and lethal activities. The haemolytic activities of magnificalytins I and II were 3.6 times  $10^{-4}$  HU/mg and 3.3 times  $10^{-4}$  HU/mg, while their LD<sub>50</sub> (i.v., mice) values were approximately 0.14  $\mu$ -g/g and 0.32  $\mu$ -g/g, respectively. The amino acid composition and N-terminal sequences of magnificalytins I and II were also obtained. They do not possess any cysteine or cystine residue, but are rich in basic and hydrophobic amino acids. The N-terminal amino acid sequences of magnificalytins I and II are ALAGTIIAGASLTFKILDEV and SAALAGTIIDGASLGFDILNKV, respectively. These are highly homologous to cytolytins from other sea anemones, particularly cytolytin III from *Stichodactyla helianthus*, a Caribbean anemone.

L4 ANSWER 32 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 29  
AN 1993:485127 BIOSIS  
DN PREV199396118727  
TI Isolation and characterization of a hemorrhagin from the venom of *Calloselasma rhodostoma* (Malayan pit viper).  
AU Ponnudurai, Gnanajothy; **Chung, Maxey C. M.**; Tan, Nget-Hong (1)  
CS (1) Dep. Biochem., Univ. Malaya, Kuala Lumpur Malaysia  
SO Toxicon, (1993) Vol. 31, No. 8, pp. 997-1005.  
ISSN: 0041-0101.

DT Article  
LA English  
AB The major hemorrhagin (termed rhodostoxin) of the venom of *Calloselasma rhodostoma* (Malayan pit viper) was purified to electrophoretic homogeneity by Sephadex G-200 gel filtration followed by high performance ion exchange chromatography. The purified hemorrhagin also yielded a single peak in reversed-phase HPLC. It had an isoelectric point of 5.3 and a mol. wt of 34,000. Rhodostoxin exhibited potent proteolytic, hemorrhagic and edema-inducing activities but was not lethal to mice at a dose of 6  $\mu$ -g/g (i.v.). Treatment of rhodostoxin with EDTA eliminated both the proteolytic and hemorrhagic activities completely. The N-terminal sequence of rhodostoxin was determined to be NHEIKRHVDIVVVXDSRFCTK.

L4 ANSWER 33 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 30  
AN 1994:28543 BIOSIS  
DN PREV199497041543  
TI Pyridylethylation of cysteine residues in proteins.  
AU Kao, Mandy C. C.; **Chung, Maxey C. M.**  
CS Bioprocessing Technol. Unit, Natl. Univ. Singapore, 10 Kent Ridge Crescent, Singapore 0511 Singapore  
SO Analytical Biochemistry, (1993) Vol. 215, No. 1, pp. 82-85.  
ISSN: 0003-2697.

DT Article  
LA English  
AB A procedure is described for using an inert polyvinylidene difluoride type membrane (e.g., ProBlott) as a matrix for on-membrane gas-phase pyridylethylation and as a desalting (cleanup) support following the solution phase pyridylethylation of cysteine residues in proteins. As a desalting support for 200 pmol of cardiotoxin, the membrane-derived sample gave a better recovery of phenylthiohydantoin (PTH)-amino acids during N-terminal sequencing compared to the sample obtained by rpHPLC. Using on-membrane gas-phase pyridylethylation, followed by replacement of glass-fiber filter disc and membrane washing/drying in vacuo, it was possible to eliminate the broad background peak that coeluted with PTH-Ala in the HPLC chromatogram completely. Using this modified procedure, there

was no evidence of N-terminal alkylation of the amino-termini of proteins.

L4 ANSWER 34 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 31  
AN 1994:37711 BIOSIS  
DN PREV199497050711  
TI Isolation and characterization of pectin methylesterase from papaya.  
AU Lim, Yin-Mei; **Chung, Max C. M.** (1)  
CS (1) Dep. Biochem., Natl. Univ. Singapore, 10 Kent Ridge Crescent,  
Singapore 0511 Singapore  
SO Archives of Biochemistry and Biophysics, (1993) Vol. 307, No. 1, pp.  
15-20.  
ISSN: 0003-9861.  
DT Article  
LA English  
AB Pectin methylesterase (PME) (EC 3.1.1.11) has been purified to apparent  
homogeneity from ripe papaya fruits. The purification protocol consisted  
of ammonium sulphate precipitation (60-80%) and cation exchange  
chromatography in CM Sepharose CL-6B and Mono S. Papaya PME consists of  
two components (PME 1 and PME 2), which have been shown to be isoenzymes  
by Ferguson plot analysis. The molecular weight of the enzyme is 27,000  
while its isoelectric point is greater than pH 9.0. The N-terminal  
sequences of PME 1 and PME 2 are SVVTPNAVVDGDFXFKTG. Both PME 1 and PME  
2 showed optimum activities at pH 8.0 and 35 degree C. The average K-mS of  
PME 1 and PME 2 are 0.0071 and 0.0166 g/liter pectin respectively, while  
the corresponding average V-maxS are 741 and 800 mu-mol methanol/min/ mg  
protein, respectively. Papaya pectin methylesterase is activated by  
cations, but the effect is more pronounced for divalent than monovalent  
cations. Inhibition studies showed that sucrose is a noncompetitive  
inhibitor while p-chloromercuribenzoic acid has no significant effect on  
its activity.

L4 ANSWER 35 OF 36 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 32  
AN 1993:8774 BIOSIS  
DN PREV199395008774  
TI Purification and characterization of a novel enzyme, arylalkyl  
acylamidase, from Pseudomonas putida Sc2.  
AU Shimizu, Sakayu (1); Ogawa, Jun; **Chung, Max Ching-Ming**; Yamada,  
Hideaki  
CS (1) Dep. Agricultural Chemistry, Kyoto University, Kitashirakawa,  
Oiwake-cho, Sakyo-ku, Kyoto, Jpn. 606  
SO European Journal of Biochemistry, (1992) Vol. 209, No. 1, pp. 375-382.  
ISSN: 0014-2956.  
DT Article  
LA English  
AB A novel enzyme, arylalkyl acylamidase, which shows a strict specificity  
for N-acetyl arylalkylamines, but not acetanilide derivatives, was  
purified from the culture broth of Pseudomonas putida Sc2. The purified  
enzyme appeared to be homogeneous, as judged by native and SDS/PAGE. The  
enzyme has a molecular mass of approximately 150 kDa and consists of four  
identical subunits. The purified enzyme catalyzed the hydrolysis of  
N-acetyl-2-phenylethylamine to 2-phenylethylamine and acetic acid at the  
rate of 6.25 mu-mol cntdot min-1 cntdot mg-1 at 30 degree C. It also  
catalyzed the hydrolysis of various N-acetyl arylalkylamines containing a  
benzene or indole ring, and acetic acid arylalkyl esters. The enzyme did  
not hydrolyze acetanilide, N-acetyl aliphatic amines, N-acetyl amino  
acids, N-acetyl amino sugars or acylthiocholine. The apparent K-m for  
N-acetylbenzylamine, N-acetyl-2-phenylethylamine and N-acetyl-3-  
phenylpropylamine are 41 mM, 0.31 mM and 1.6 mM, respectively. The  
purified enzyme was sensitive to thiol reagents such as Ag-2SO-4, HgCl-2  
and p-chloromercuribenzoic acid, and its activity was enhanced by divalent  
metal ions such as Zn-2+, Mg-2+ and Mn-2+.

L4 ANSWER 36 OF 36 CAPLUS COPYRIGHT 2001 ACS DUPLICATE 33  
AN 1988:586033 CAPLUS  
DN 109:186033  
TI Ketopantoic acid reductase of Pseudomonas maltophilia 845. Purification,  
characterization, and role in pantothenate biosynthesis  
AU Shimizu, Sakayu; Kataoka, Michihiko; **Chung, Max Ching Ming**;  
Yamada, Hideaki



CS Dep. Agric. Chem., Kyoto Univ., Kyoto, 606, Japan  
 SO J. Biol. Chem. (1988), 263(4), 12077-84  
 CODEN: JBCHA3; ISSN: 0021-9258  
 DT Journal  
 LA English  
 AB Ketopantoate reductase (EC 1.1.1.169) (I), that catalyzes the formation of D-(-)-pantoic acid (II) from ketopantoic acid (III), was purified 6000-fold to apparent homogeneity with a 35% overall recovery from P. maltophilia 845 and then crystd. The mol. wt. of native I, as estd. by the sedimentation equil. method, was 87,000, and the subunit mol. wt. was 30,500. I showed high specificity for III as a substrate ( $K_m = 400 \mu\text{M}$ ,  $V_{max} = 1310 \text{ units/mg protein}$ ) and NADPH as a coenzyme ( $K_m = 31.8 \mu\text{M}$ ). Only 2-keto-3-hydroxyisovalerate ( $K_m = 8.55 \text{ mM}$ ,  $V_{max} = 35.8 \text{ units/mg}$ ) was reduced among a variety of other carbonyl compds. tested. The reaction was reversible ( $K_m$  for II =  $52.1 \text{ mM}$ ), although the reaction equil. greatly favored the direction of II formation. That I was responsible for II synthesis necessary for the biosynthesis of pantothenic acid in P. maltophilia 845 was indicated by the observation that only this enzyme was missing in II- (or pantothenate)-requiring mutants derived from P. maltophilia 845 among several enzymes [e.g., ketopantoyllactone reductase (EC 1.1.1.168) and acetohydroxy acid isomeroreductase (EC 1.1.1.86)], which may be concerned in the formation of II, assayed, whereas it was present in substantial amts. in the parent strain and in spontaneous revertants of the mutants.

=> e lim renee/au

E1	4	LIM REGINA/AU
E2	1	LIM REMEDIOS A/AU
E3	0 -->	LIM RENEE/AU
E4	4	LIM RENEE L H/AU
E5	2	LIM RENEE LAY HONG/AU
E6	1	LIM REX/AU
E7	5	LIM REX W/AU
E8	3	LIM RICARDO/AU
E9	24	LIM RICHARD/AU
E10	1	LIM RICHARD A/AU
E11	1	LIM RICHARD H K/AU
E12	3	LIM RICHARD M/AU

=> s e4-e5

L5 6 ("LIM RENEE L H"/AU OR "LIM RENEE LAY HONG"/AU)

=> dup rem l5

PROCESSING COMPLETED FOR L5

L6 3 DUP REM L5 (3 DUPLICATES REMOVED)

=> d bib ab 1-

YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1  
 AN 2000:337702 BIOSIS  
 DN PREV200000337702  
 TI Cloning and expression of immunoreactive antigens from Mycobacterium tuberculosis.  
 AU Lim, Renee Lay Hong (1); Tan, Li Kiang; Lau, Wai Fun; Chung, Maxey Ching Ming; Dunn, Roseanne; Too, Heng Phon; Chan, Lily  
 CS (1) Bioprocessing Technology Centre, National University of Singapore, 10 Kent Ridge Crescent, 5th Floor, MD11, Singapore, 119260 Singapore  
 SO Clinical and Diagnostic Laboratory Immunology, (July, 2000) Vol. 7, No. 4, pp. 600-606. print.  
 ISSN: 1071-412X.  
 DT Article  
 LA English

SL English  
AB Four immunoreactive proteins, B.4, B.6, B.10, and B.M, with molecular weights ranging from 16,000 to 58,000, were observed from immunoblots of Mycobacterium tuberculosis total lysates screened with sera from individuals with active tuberculosis. These proteins were identified from microsequence analyses, and genes of proteins with the highest homology were PCR amplified and cloned into the pQE30 vector for expression studies. In addition, a 37.5-kDa protein, designated C17, was identified from a phage expression library of M. tuberculosis genomic DNA. Preliminary immunoblot assays indicated that these five resultant recombinant proteins could detect antibodies in individuals with active pulmonary and extrapulmonary tuberculosis. The overall ranges of sensitivities, specificities, positive predictive values, and negative predictive values for the recombinant antigens were 20 to 58, 88 to 100, 69 to 100, and 56 to 71%, respectively. The B.6 antigen showed preferential reactivity to antibodies in pulmonary compared to nonpulmonary tuberculosis serum specimens. All of these recombinant antigens demonstrated potential for serodiagnosis of tuberculosis.

L6 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 2  
AN 1998:92898 BIOSIS  
DN PREV199800092898  
TI Nucleotide variation in the cytidine triphosphate synthetase gene of Giardia duodenalis.  
AU Swarbrick, Alexander; Lim, Renee L. H.; Upcroft, Jacqueline A.; Stewart, Thomas S. (1)  
CS (1) Sch. Biochem. Mol. Genetics, Univ. NSW, Sydney, NSW 2052 Australia  
SO Journal of Eukaryotic Microbiology, (Nov.-Dec., 1997) Vol. 44, No. 6, pp. 531-534.  
ISSN: 1066-5234.  
DT Article  
LA English  
AB The cytidine triphosphate synthetase genes from three diverse strains of Giardia duodenalis have been sequenced and found to vary significantly from one another. The isolates were chosen as representatives of three demes as determined by several criteria including divergence in the rDNA repeat unit. Inserts in the genes and protein are conserved in length but are the most divergent regions among the three sequences examined. Variation in the rest of the gene occurs primarily in the third base position resulting in many silent mutations. One of the isolates (1709) was found to contain two genes with high sequence homology.

L6 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 3  
AN 1996:380165 BIOSIS  
DN PREV199699102521  
TI Isolation, characterization and expression of the gene encoding cytidine triphosphate synthetase from Giardia intestinalis.  
AU Lim, Renee L. H.; O'Sullivan, William J.; Stewart, Thomas S. (1)  
CS (1) Sch. Biochem. Mol. Genet., Fac. Biol. Behavioural Sci., Univ. N.S.W., Sydney, NSW 2052 Australia  
SO Molecular and Biochemical Parasitology, (1996) Vol. 78, No. 1-2, pp. 249-257.  
ISSN: 0166-6851.  
DT Article  
LA English  
AB The cytidine triphosphate synthetase gene from Giardia intestinalis was cloned using a PCR-based strategy. A 519 bp PCR product was obtained from the amplification of genomic DNA using two oligonucleotides derived from the CTP synthetase amino acid consensus sequences DPYINVDPG and KTKPTQ. This product was used to probe restriction endonuclease digested genomic DNA and the respective plasmid mini-libraries. Two genomic clones were obtained one with a 3.6 kb HindIII DNA fragment, containing approximately three-quarters of the 5'-end of the synthetase gene and subsequently, a 5.8 kb PstI DNA fragment which contained the whole gene. The intronless gene has a 1863 bp open reading frame encoding 620 amino acids (M-r of 68.3 kDa). A well conserved catalytic glutamine aminotransferase (GAT) domain was identified. In addition, three insert sequences were found which are not present in CTP synthetase from other species. Alignment and comparison of the deduced amino acid sequence relative to CTP synthetases

from other species revealed a high degree of identity (34%) with a greater resemblance to prokaryotes than eukaryotes. The gene is located on chromosome 6 and the messenger RNA encoding it is estimated to be 1.9 kb. The coding region of *G. intestinalis* CTP synthetase was generated by PCR and subsequently cloned into the pQE30 vector for expression in *E. coli*. This construct yielded a soluble and enzymatically active recombinant protein which was purified by a Ni-NTA affinity column. The purified recombinant protein had a subunit molecular weight of 69.5 kDa and a native molecular weight of approximately 274 kDa. Kinetic studies of the partially purified recombinant *G. intestinalis* CTP synthetase gave apparent  $K_m$  values of 0.1 mM and approximately 0.5 mM for the substrates UTP and L-glutamine respectively in accord with previously reported values